

Comprehensive Overview of the Structure and Regulation of the Glucocorticoid Receptor

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Glucocorticoids are among the most prescribed drugs worldwide for the treatment of numerous immune and inflammatory disorders. They exert their actions by binding to the glucocorticoid receptor (GR), a member of the nuclear receptor superfamily. There are several GR isoforms resulting from alternative RNA splicing and translation initiation of the GR transcript. Additionally, these isoforms are all subject to several transcriptional, post-transcriptional, and post-translational modifications, all of which affect the protein's stability and/or function. In this review, we summarize recent knowledge on the distinct GR isoforms and the processes that generate them. We also review the importance of all known transcriptional, post-transcriptional, and post-translational modifications, including the regulation of GR by microRNAs. Moreover, we discuss the crucial role of the putative GR-bound DNA sequence as an allosteric ligand influencing GR structure and activity. Finally, we describe how the differential composition and distinct regulation at multiple levels of different GR species could account for the wide and diverse effects of glucocorticoids. (*Endocrine Reviews* 35: 671–693, 2014)

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I. Introduction

Glucocorticoids (GCs) are steroid hormones that are produced by the adrenal cortex, under tight regulation by the hypothalamic-pituitary-adrenal gland axis.

GCs are pivotal regulators of a wide variety of fundamental processes, such as metabolic homeostasis, cell proliferation, inflammation, immune responses, development, and reproduction (1–3). Because of their lipophilic nature, GCs can readily diffuse across cellular membranes to exert their biological actions.

GCs function by binding to their intracellular receptor, the GC receptor (GR), which is a ligand-inducible transcription factor belonging to the nuclear receptor superfamily (4). In the absence of ligand, GR resides predominantly in the cytoplasm within a multimeric chaperone complex comprising heat-shock protein (hsp) 90, hsp70, hsp90-binding protein p23, immunophilins (eg, FKBP51, FKBP52, Cyp44, and PP5), and other factors to prevent its degradation and assist in its maturation (5). Upon ligand binding, the GR complex changes its conformation and travels to the nucleus, where it interacts with coregulators that assist GR transcriptional actions. The SRC family of coactivators are well known to aid GR in its transcrip-

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Abbreviations: AF1, activation function 1; AP-1, activator protein-1; ARE, AU-rich element; AU, adenylate uridylylate; CDK, cyclin-dependent kinase; ChIPseq, chromatin immunoprecipitation sequencing; DBD, DNA-binding domain; GBS, GR binding sequence; GC, glucocorticoid; GR, GC receptor; GRE, GC response element; GSK-3 β , glycogen synthase kinase 3 β ; HDAC, histone deacetylase; hGR, human GR; hsp, heat-shock protein; JNK, c-Jun N-terminal kinase; LBD, ligand-binding domain; miR, micro-RNA; NF κ B, nuclear factor κ B; nGRE, negative GRE; NLS, nuclear localization signal; NO, nitric oxide; NRS, nuclear retention signal; NTD, N-terminal domain; PTM, post-translational modification; RA, rheumatoid arthritis; Ser, serine; SUMO-1, small ubiquitin-related modifier-1; Tyr, tyrosine; UTR, untranslated region.

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tional activity, but viral components, for example, have also been described to act as coactivators of GR (6). On the contrary, other GR interaction partners can act as negative regulators of GC signaling. For instance, the interaction of GR with 14–3–3 σ enhances GR nuclear export (7), binding to Fas associated protein with death domain-Like interleukin-1 β converting enzyme-Associated Huge protein (FLASH) impairs GR transcriptional actions due to reduced availability of the GR interacting protein 1 coactivator (8), and binding of GR with β -units of G proteins disrupts the transcriptional activation complex (9). Note that GR is not rigidly compartmentalized; a constant shuttling between nucleus and cytoplasm occurs with both activated and nonactivated forms of GR (5, 10), a mechanism that allows the GR to dynamically act as a sensor. GR is constitutively expressed in virtually every cell type, but tissue-specific expression patterns of GR result in different transcriptional outcomes (11, 12).

In the nucleus, GR acts as a transcription factor, mediating the up- and down-regulation of numerous genes in a coordinated fashion. Transcriptional induction of genes by GR is mediated mainly by binding of GR dimers to so-called GC response elements (GREs). Note that this GR-mediated transcriptional profile is very cell type-specific, as was recently confirmed by several chromatin immunoprecipitation sequencing (ChIPseq) analyses in which limited overlap in GR binding sites in different cell types and tissues could be found (13–15). In addition to the induction of genes encoding anti-inflammatory proteins, such as *Tsc22d3* (encoding GILZ) and *Dusp1* (encoding MKP-1) (16), GR exerts part of its anti-inflammatory effects by interacting with proinflammatory transcription factors, such as nuclear factor κ B (NF κ B) and activator protein-1 (AP-1), leading to the transcriptional repression of many genes. In addition to ligand-activated GR, a recent report shows gene expression changes by the unliganded GR (17). Although most activities of GR are performed in the nucleus, rapid non-genomic GC actions also occur, which can indirectly affect gene transcription, eg, by direct inactivation of MAPK or phosphatidylinositol-3-kinase (18). Moreover, GCs can intercalate into cellular membranes and alter the physicochemical properties of these membranes, as well as the activities of membrane-associated proteins (19, 20). GR itself can also interact with cellular membranes, eg, a membrane-bound GR was identified as a component of the T-cell receptor complex, negatively affecting downstream signaling (21). Altogether, these mechanisms in part support the rapid anti-inflammatory effects of GCs (22).

In this review, we provide a state-of-the-art overview of the structure and regulation of GR. We discuss both transcriptional and post-translational modifications. Further-

more, we highlight the importance of recently identified micro-RNAs (miRs) in the regulation of GR production and activity. We also focus on the key novel findings about the allosteric aspect of the DNA sequence in directing GR structure and transcriptional outcome. We emphasize that the regulation of GR, and hence its entire signaling cascade, is enormously complex, and that understanding this advanced regulation is crucial for the development of more effective GC therapies.

II. Regulation of GR Gene Expression

GR levels within the cell are not static but are tightly controlled by numerous factors and at multiple levels. In eukaryotic cells, protein expression levels in general are primarily determined by the mRNA level. Gene expression is firmly controlled by a wide variety of mechanisms at transcriptional, post-transcriptional, and post-translational levels. Although GR is constitutively expressed, it shows distinct expression patterns in different cells and tissues due to differential regulation. Here, we describe the multilevel regulatory mechanisms, leading to its tissue-specific expression profile. For a complete overview, see Figure 1.

A. Genomic Structure of GR

In humans, the GR gene (*NR3C1*) is located on the short arm of chromosome 5 (5q31Y32) (23). This gene comprises nine exons, of which exons 2 through 9 encode the protein. So far, 13 variants of human GR (hGR) exon 1 differing in the upstream promoter regions (promoters A through F and H through J) have been identified (Figure 1) (24–28). Both promoters A and C regulate three distinct untranslated exons (1A_{1–3}, 1C_{1–3}) owing to the unique promoter fragments (25). The promoter region is long and includes elements as far upstream as 35 kb. The location of the different promoters was estimated based on the presence of highly conserved regions and on literature mining (25, 29). For example, the hGR1A promoter regulating transcription starting from exon 1C was found about 27 kb upstream of that transcription start site (25).

The existence of these alternative promoters, each displaying a distinct level of expression and tissue specificity, illustrates the plasticity and complexity in hGR regulation at the transcriptional level. The 5' untranslated region (UTR) of hGR is very similar to the mouse and rat first exons, at least in number and structure (24, 30).

B. Transcriptional control of the GR-coding gene *NR3C1*

The promoter region (5' UTR) of GR has been extensively studied. It is very GC-rich (72% GC content) but does not contain a TATA or a CAT box (29, 31). The

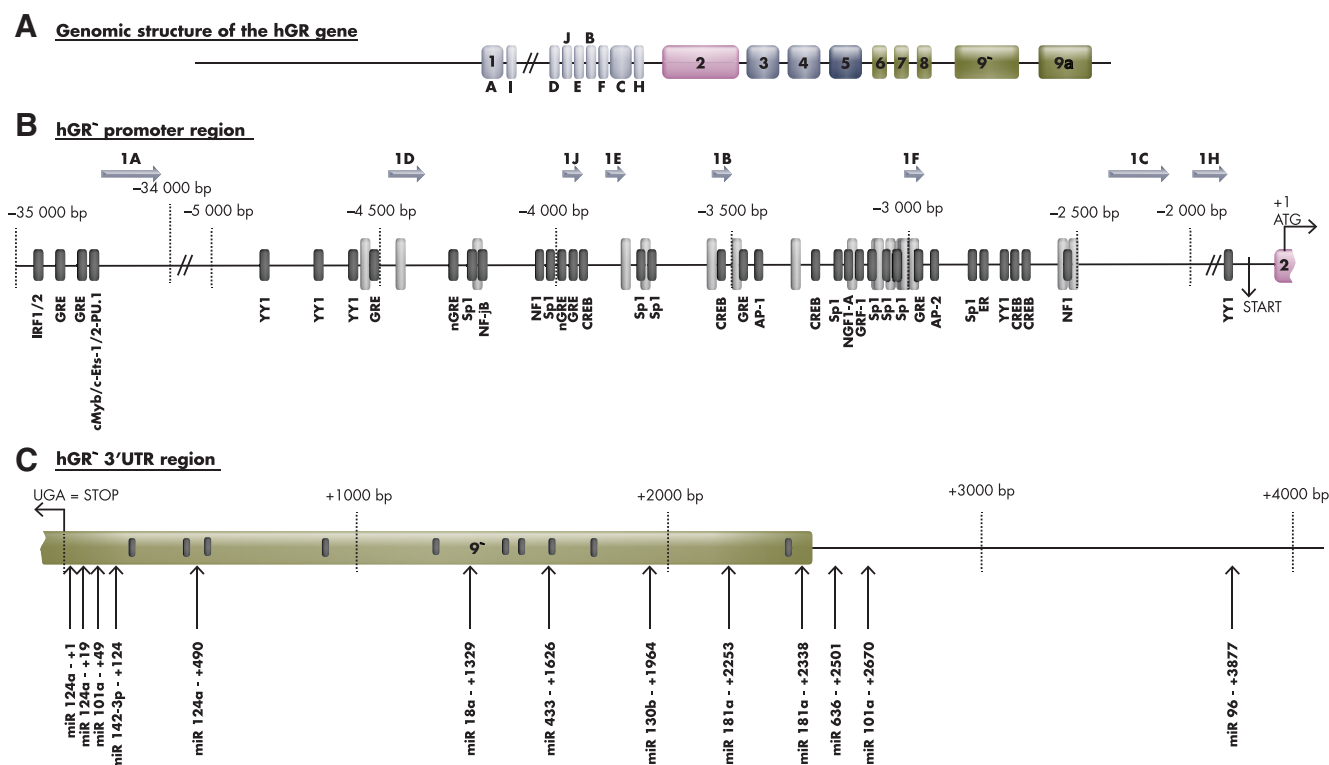
Figure 1.

Figure 1. Regulation of GR gene expression. A, Genomic structure of the hGR gene. B, hGR α promoter region: transcription factor binding sites are depicted in dark gray boxes and GC-rich regions in light gray boxes. C, hGR α 3' UTR region: AU-rich elements are depicted in dark gray boxes and miR target sites are indicated by the arrows.

major transcription start site is located in exon 2, 134 bp upstream of the ATG initiation codon (31). The promoters comprise multiple binding sites for several known transcription factors: AP-1 (29, 32), AP-2 (33), NF κ B (34), estrogen receptor (29, 35), cAMP response element binding protein (29), NF1/CTF1 (29), Yin Yang 1 (36), Sp1 (27, 33, 36), IRF1/2 (25, 37), cMyb (38, 39), PU.1 (38, 39), and EGR1/NGF1-A (40–42). Whereas the above-mentioned transcription factors are known to up-regulate GR expression, GC responsive factor-1 (43, 44) and c-Ets-1/2 (38) have been shown to repress GR expression. Note that small noncoding “tiny RNAs” might also be involved in the transcriptional regulation of GR because these RNA molecules can turn off target gene expression by binding to complementary regions in promoter DNA (45). However, this hypothesis needs further investigation and validation.

Moreover, multiple GREs and negative GREs (nGREs) have been identified in the promoter region of GR (46). This implies that GR can specifically bind to its own promoter. Several GRE half-sites act in concert with other transcription factors, such as cMyb and c-Ets-1/2 (38). In the presence of cMyb, the GC-activated GR is recruited to the promoter of its own gene and up-regulates its own

expression. The interaction of GR with family members of the c-Ets family leads to repression of GR expression (38, 39). Additionally, a functional nGRE was recently identified in exon 6 of GR (47). This nGRE might contribute to the ligand-dependent homologous down-regulation of GR. Consequently, chronic administration of GCs can constitutively repress GR expression via an autoregulatory loop and thereby induce GC resistance, ie, unresponsiveness to the beneficial anti-inflammatory effects of GR. It is clear that the binding of different transcription factors to the promoter region of the GR gene (*NR3C1*) can have different effects. This could explain the tissue-specific effects of GCs, but the mechanisms at work in this promoter in different cell types are still far from clear. Moreover, autocrine regulation of GR further complicates the understanding of GR biology.

C. Post-transcriptional regulation of the GR transcript

The stability of GR mRNA is controlled by various mechanisms. The highly overrepresented presence of adenylate uridylylate (AU)-rich elements (AREs) in the 3' UTR of GR mRNA might mediate mRNA destabilization and hence affect GR protein expression (48, 49). However, the

mechanisms and contributions of ARE-induced GR mRNA degradation are not fully understood or experimentally evaluated. One could make use of ARE databases, such as AREsite and ARED 3.0, to search for additional ARE sites and study them *in silico* (50, 51), but such an endeavor has not been reported.

miRs present another level for fine-tuning GR levels. Several reports have illustrated the importance of miRs in controlling GR mRNA stability. miRs were discovered in 2001 (52, 53) and later predicted to regulate up to 30% of all genes (54). Since then, various studies have led to the conclusion that miRs account for about 1% of the entire human genome and play critical roles in a wide variety of cellular processes, such as inflammation, cell differentiation, and apoptosis (55). miRs are single-stranded non-coding RNA molecules of about 21 nucleotides. They exert their functions by incomplete base pairing to sequence motifs in their target mRNAs, preferentially in the 3' UTR, and in that way they interfere with mRNA stability and translation (56). Potential miRs targeting the 3' UTR of GR can be predicted using publicly available software packages, such as Targetscan 4.2 and MiRwalk (57, 58). These programs indicate that the 3' UTR of GR is indeed under tight miR control. Moreover, several reports have illustrated the targeting of the GR 3' UTR by miRs. For example, a miR microarray analysis has identified adrenal miRs that seem to target GR upon ACTH stimulation, of which four were experimentally confirmed to target the 3' UTR of GR, namely miR-96, miR-101a, miR-142-3p, and miR-433. These miRs can repress the GR expression level to 40% (59). Because these miRs are up-regulated upon ACTH stimulation and subsequently repress GR expression levels, this could contribute to the GC-induced autoregulation of GR. Furthermore, the decreased GR level in human T-cell acute lymphoblastic leukemia could point to an oncogenic role for miR-142-3p because the GC-resistant condition could be reverted by a miR-142-3p inhibitor (60). The above-mentioned microarray analysis also identified another differentially expressed miR, miR-18a (59). Interestingly, miR-18a has already been shown to be increased in the paraventricular nucleus of rats, where it mediates the down-regulation of GR (61). Furthermore, the importance of GR regulation by miR-18, which is widely expressed throughout the body, has also been illustrated by Vreugdenhil et al (62). miR-124a, which is predominantly expressed in the brain, was also shown to down-regulate GR expression. Both miRs bind to the 3' UTR of GR, and in that way they mediate the down-regulation of GR, which leads to attenuation of GR-mediated transcriptional induction (62). Another report has linked the expression of miR-124 to the subsequent down-regulation of GR with acquired GC resistance in

sepsis patients. Furthermore, the expression of miR-124 was up-regulated by GC-induced stimulation of T cells. This is another possible mechanism for the GC-induced autoregulation of GR and acquisition of GC resistance in sepsis. Actually, T cells of sepsis patients exhibit a slight decrease in GR expression, correlating with slightly increased miR-124 expression (63). Another recent report indicates a critical role for miR-130b, miR-181a, and miR-636 in regulating the GC response in multiple myeloma (64).

These findings suggest that miRs can, by post-transcriptional regulation, affect the expression levels of GR and its subsequent activity. Moreover, the modulation of GR translation activity by miRs could be responsible for development of GC resistance in response to GC treatment of various diseases. Thus, elucidating the control of GR levels by miRs is fundamental for understanding the etiology of several proinflammatory and autoimmune disorders and the associated reduced GC response, as well as for research on cancer and neurological conditions, such as anxiety and depression.

III. Splice Variants and Isoforms of GR

The traditional view that GCs exert their functions by binding to one single GR protein has changed during recent years after identification of several GR isoforms (Figure 2). These subtypes are the result of alternative processing of the primary mRNA in terms of splicing and translation. The isoforms differ in their expression patterns, gene regulatory networks, and other functional aspects. Furthermore, differential post-translational modifications (PTMs) increase the variety of GR subtypes even more.

A. Alternative mRNA splicing

Alternative splicing near the 3' UTR of the primary hGR transcript generates two isoforms (hGR α and hGR β) differing at their C termini. The classical hGR α protein contains the end of exon 8 joined to exon 9 α , whereas in the splice variant hGR β , which results from an alternative splice acceptor site, exon 8 is joined to the more downstream exon 9 β (65–67). These two isoforms share identical amino acids up to position 727. The C-terminal ends differ: hGR α contains an extra 50 amino acids, resulting in a protein of 777 amino acids, whereas hGR β contains 15 additional amino acids and hence is composed of 742 amino acids. Hence, hGR β has a shortened ligand-binding domain (LBD) that cannot bind GCs. Nevertheless hGR β , which is constitutively found in the nucleus, is transcriptionally active. The GR antagonist mifepristone (RU486)

Figure 2.

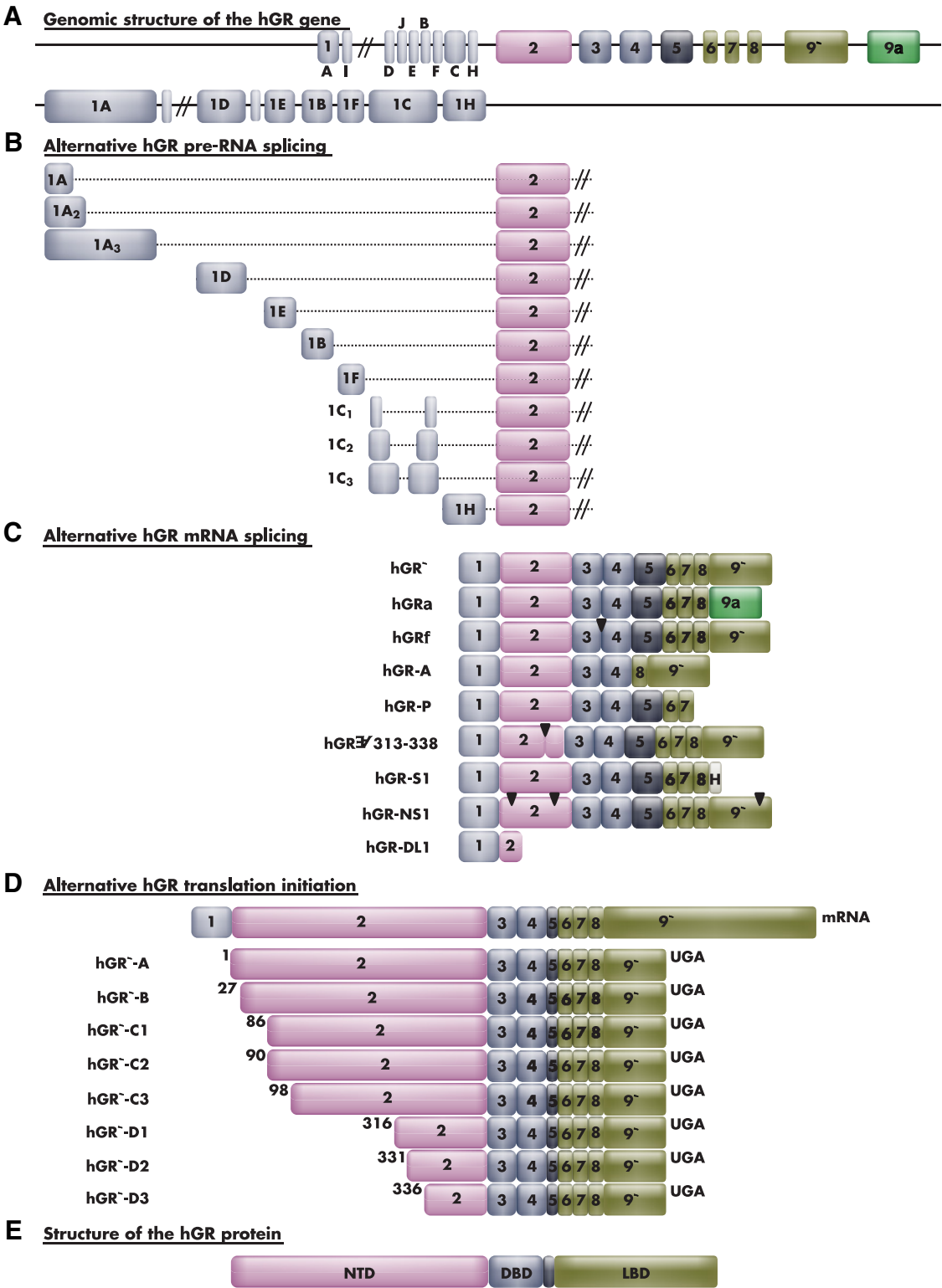


Figure 2. Splice variants and isoforms of GR. A, Genomic structure of the hGR gene. B, Alternative hGR pre-RNA splicing. C, Alternative hGR mRNA splicing; black triangles depict deletions. D, Alternative hGR translation initiation. E, Structure of the hGR protein.

can bind to it (68), but the endogenous ligand is currently unknown. Genome-wide expression analyses have shown that hGR β can directly induce and repress a large number of genes that are not controlled by GR α (65, 68, 69). By recruitment of histone deacetylase corepressors, hGR β can mediate transcriptional repression (71). In addition to this intrinsic gene regulatory profile, transcriptional regulation by hGR β can also depend on its hGR α antagonism. Indeed, when coexpressed with hGR α , hGR β can function as a dominant negative splice variant of hGR α , inhibiting its activity (72–74). In this way, hGR β can cause GC resistance. Indeed, several reports prove the contribution of hGR β to many diseases and to resistance to GC therapies (34). For example, bronchiolitis patients infected with respiratory syncytial virus showed an increase in GR β expression correlating with disease severity (75). Moreover, elevated GR β levels were found in a variety of GC-resistant disorders, such as asthma, rheumatoid arthritis (RA), and inflammatory bowel disease (76–78).

In addition, increased GR β levels also contribute to the development of erythrocytosis in patients with polycythemia vera or eosinophilic chronic rhinosinusitis (79, 80). It was initially believed that GR β is found only in humans, but a recent report illustrated the existence of GR β in mouse (mGR β) and rat as well as its prominent role in metabolism. Although mGR β arises from differential splicing using intron 8 rather than exon 9 as in humans, it is similar in structure and functionality to its counterisoform, hGR β (81, 82).

Additional hGR isoforms resulting from alternative splicing have been identified: 1) hGR γ , a widely expressed splice variant, which includes an insertion of a single arginine residue between the two zinc fingers in the DNA-binding domain (DBD) (83–85); 2) hGR-A, lacking exons 5 through 7 (86, 87); 3) hGR-P, a truncated isoform missing exons 8 and 9 (86, 87); 4) hGR δ (hGR Δ 313–338), a splice variant with a deletion in exon 2; 5) hGR-S1, retaining intron H between exons 8 and 9 (88); 6) hGR-NS1, a variant containing three nonsynonymous single-nucleotide polymorphisms; and 7) hGR-DL1, a truncated isoform due to a single nucleotide deletion in exon 2. The latter three were identified only recently (89). hGR γ and hGR α bind GCs and DNA with similar affinities. However, the ability of hGR γ to stimulate GRE reporters is compromised, and so it has a different transcriptional profile from the classical GR α isoform (83, 84). Moreover, GR γ expression has been associated with GC resistance in small-cell lung carcinoma, corticotrope adenomas, and childhood acute lymphoblastic leukemia (83, 84, 90).

Little is known about GR-A, but studies on GR-P provide evidence for its expression in several tissues. Furthermore, GR-P seems to be the predominant GR subtype in several

GC-insensitive cancer cell types (86, 87, 91). Next, hGR Δ 313–338 or hGR δ is expressed in several tissues, such as lung, sc adipose tissue, liver, skin, heart muscle, and hippocampus. Interestingly, the deleted region has a number of potential phosphorylation sites important for the transactivation potential of hGR. Therefore, it was suggested that this variant exhibits an altered GC-induced transactivation profile (92). hGR-S1 has an early termination site due to the preservation of the last intron, which contains a stop codon. Hence, this splice variant gives rise to a truncated protein of 745 amino acids and has a lower transactivation potential than the classical GR α isoform, probably due to weaker ligand binding (88). Also, little is known about the recently identified hGR-NS1 and hGR-DL1. However, the activity of hGR-NS1 is at least double that of hGR α , whereas the activity of hGR-DL1 is only 10% of the activity of hGR α (89). These latter three isoforms have not been linked with inflammatory disorders or GC-resistant diseases (Figure 2).

B. Alternative translation initiation

The complexity of GR biology is complicated even further by alternative translation initiation. The eight alternative initiation sites (AUG start codons) in exon 2 result in eight hGR α subtypes with truncated N-terminal domains (NTDs), named hGR α -A to D (A, B, C1, C2, C3, D1, D2, and D3) (93–95). These N-terminal isoforms are the product of both leaky ribosomal scanning and ribosomal shunting (94). These distinct initiation sites are conserved in other species, such as monkey, rat, and mouse. hGR α -A forms the classical full-length hGR protein of 777 amino acids, which is generated from the first initiator codon (methionine +1) (Figure 2).

All these hGR α subtypes have tissue-specific expression patterns that are conserved in different species. For example, levels of the GR α -C isoforms are significantly higher in the pancreas and colon, whereas levels of GR α -D are highest in spleen and lungs. Also, levels of GR α -B are higher than those of GR α -A in liver and thymus. These GR α isoforms display different transcriptional activities in response to GCs, with a unique gene induction profile, and have diverse subcellular distributions (94). For example, GR α -C isoforms have enhanced activity, at least in the induction of proapoptotic genes, whereas the GR α -D subtypes show much lower activity compared to hGR α -A, or even none at all (96–98). Additionally, the GR α -C isoforms are also more efficient in recruiting coactivators, including RNA polymerase II, than the GR α -D isoforms, probably due to the variable NTD (97). Increased expression levels of the truncated GR α -D isoforms were reported in schizophrenic patients (99). Also, the GR α -C isoforms have a subcellular distribution similar to that of GR α -A, but they have stronger transcriptional activity, suggesting the existence of an inhibitory domain within

the N-terminus of GR. Interestingly, it was also shown that increased amounts of GR-C3 enhanced GR-A transcriptional activity (93). Thus, depending on their inherent activities and relative expression in different tissues, the GC signals might be differentially transduced by the presence of variant N-terminal GR isoforms.

Although it is not illustrated in Figure 2, each of the splice variants is also expected to contain these different translation initiation sites and hence to give rise to a similar set of translational isoforms (30). Furthermore, the cellular composition of GR translational isoforms is signal- and time-dependent (35). Altogether, this wide variety of GR isoforms can explain the cellular heterogeneity and tissue-specific effects of GCs. Nevertheless, the complexity and diversity of GR isoforms is far from fully explored and warrants further study.

IV. Structure of the GR Protein

The hGR (777 amino acids) is a modular protein organized into three major functional domains with distinct functions (Figure 3). The first N-terminal 421 amino acids of the hGR protein are designated as the NTD. This do-

main contains the ligand-independent constitutive transcriptional activation function 1 (AF1), which is required for maximal transcriptional activation of GR (101, 102). AF1 is rich in acidic amino acids and is important for initiation of transcription, ie, interaction of GR with coregulators, chromatin modulators, and the basal transcription machinery (103, 104). The NTD is the most variable domain among the nuclear receptors in different species (104, 105). The next 65 amino acids in the central region of the GR comprise the DBD, which consists of two highly conserved zinc fingers. These two zinc finger motifs tetrahedrally coordinate a zinc atom held by four cysteine residues. Obviously, the DBD is required for its DNA-binding specificity, but it also plays a role in GR dimerization and interaction with cofactors or other transcription factors, such as c-Jun. More specifically, the DBD contains amino acids responsible for the binding of GR to GREs. These amino acids are primarily found in the first zinc finger, which contains the proximal (P) box responsible for the site-specific recognition of GREs. In addition, the second zinc finger in the DBD holds a specific region, the distal (D) box, comprising five amino acids required for GR homodimerization at the GRE (106–108). Interestingly, it was shown that Jun dimerization protein-2, a

small bZIP protein known to interact with the DBD of the human progesterone receptor, also interacts with the DBD of GR. Moreover, the Jun dimerization protein-2-GR interaction induces a compact structure in the NTD/AF1 in a way that facilitates the interaction of AF1 with coregulators and hence increases the transcriptional activity of GR (109). The DBD is separated from the C-terminal LBD by a hinge region encompassing amino acids 486–526. This hinge region provides GR dimers with structural flexibility that facilitates the interaction of GR dimers with their respective GRE, directed by the second zinc finger. The LBD at the C terminus is 251 amino acids long and is moderately conserved among species. A ligand-dependent transcriptional AF2 domain is embedded in the LBD (110). Additionally, the LBD also plays a role in interactions with (co-)chaperones, coregulators, and other transcription factors, and interestingly also in GR dimerization (110, 111). Fur-

Figure 3.

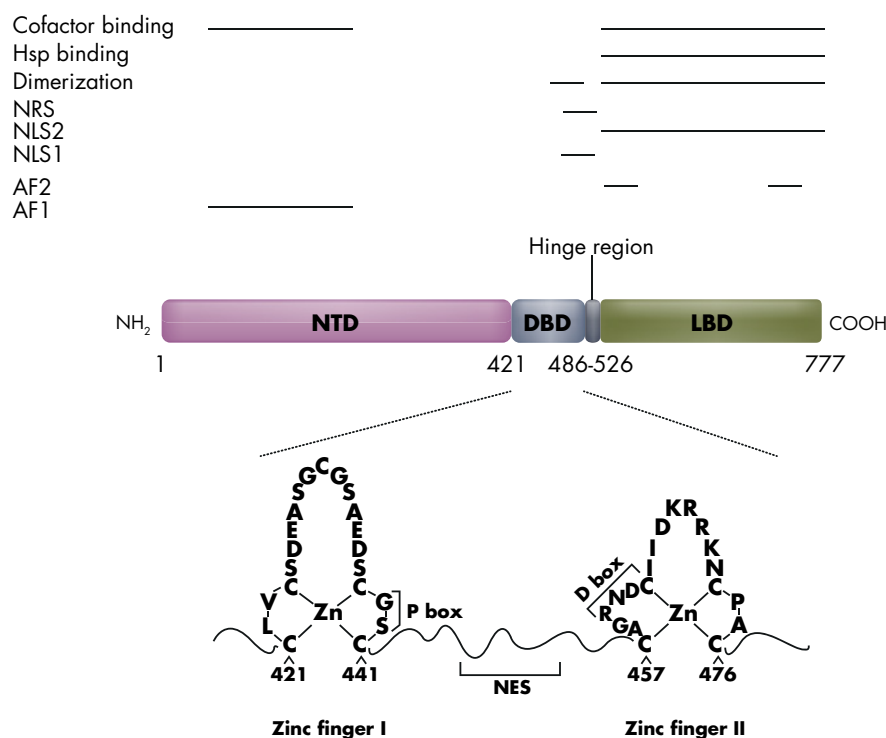


Figure 3. Structure of the GR protein. GR is composed of an NTD, a DBD consisting of two zinc fingers, a hinge region, and a C-terminal LBD. Variation in the function of GR can be correlated with these domains. P, proximal; D, distal.

thermore, nuclear localization signals (NLS1 and NLS2) are also located in the DBD and LBD, respectively (112). In addition, a nuclear export signal of 15 amino acids is located in the DBD between the two zinc fingers and mediates nuclear export of GR (113, 114). Furthermore, a nuclear retention signal (NRS) overlaps with NLS1. The NRS delays the nuclear export of GR and hence improves the transcriptional activity of GR (115).

V. GR Mutations and Polymorphisms

Mutations and polymorphisms in the hGR gene are another level of complexity influencing the tissue specificity of GCs and the molecular mechanisms of GR action. Polymorphisms in the coding and regulatory regions of *NR3C1* are associated with the response to GCs, which may be either positive (GC hypersensitivity) or negative (resistance) (116–119). Subsequently, genetic variation affects the success of GC therapy and disease pathology (120). Numerous pathological mutations have been identified in the human *NR3C1* gene (116, 117, 121–124). Elucidating the mechanisms and effects of these mutations can provide more insights into the molecular mechanisms of hGR activity and contribute to development of GC therapy for a wide variety of diseases. For an overview of the known mutations in the hGR gene and their effects, see Table 1 (only the mutations linked with a detected phenotype are listed). However, the most extensively studied mutations will be described more thoroughly, namely the BclI, N363S, and ER22/23EK polymorphisms.

Polymorphisms in the NTD of GR have been associated with variations in its mRNA levels and in its function, generally leading to increased GR transcriptional activity. It is known that the NTD, containing a transactivation domain, forms a helix upon binding with transcription factors, such as TBP (TATA box binding protein) (125), and mutations that affect helix formation can influence the transactivation activity (126). Hypothetically, a change in hydrophobicity in the NTD might alter the potential to interact with coregulatory proteins and in this way impact the transcriptional activity of GR. For example, the N363S polymorphism, located in the NTD, is associated with increased sensitivity to GCs (127, 128). Patients carrying this mutation have a higher body mass index (129), lower bone mineral density, obesity (129–133), bronchial asthma (134), bilateral adrenal incidentalomas (135), type 2 diabetes (136), and coronary artery disease (132). Like BclI carriers, patients carrying the N363S mutation also have a lower risk of developing RA (137). Generally, mutations resulting in increased GR transcriptional activity lead to an increased risk for met-

abolic side effects. On the contrary, GR insensitivity is associated with a more favorable metabolic profile.

Two linked, single-nucleotide mutations in exon 2 cause another polymorphism: a CAG to GAA mutation in codon 22, which does not result in an amino acid change (glutamic acid [E]), and a AGG to AAG mutation in codon 23, which causes substitution of arginine (R) by lysine (K) (ER22/23EK) (123, 124, 138). This polymorphism has been reported to reduce sensitivity to GCs (128). Indeed, decreased GR activity has been shown by reporter assays and expression of endogenous genes (122, 139, 140). Hence, patients carrying the ER22/23EK polymorphism display relative GC resistance (123). Carriers of the ER22/23EK polymorphism have lower risks for GC-associated side effects and therefore a lower tendency to develop type 2 diabetes or cardiovascular disease (141). Moreover, carriers have a lower risk of developing dementia (123, 142) and insulin resistance (143). However, they have an increased risk of developing RA (137) and multiple sclerosis (144).

So far, two mutations have been discovered in the DBD (V423A and V477H), both of which negatively affect GC signal transduction (145, 146). Because the DBD is crucial for proper nuclear translocation, dimerization, and DNA binding of GR (Figure 3), both mutations were described to impair the ability of GR to translocate to the nucleus and to bind to target GRE elements (145, 146).

Based on the important functions of the LBD, ie, ligand binding, nuclear translocation, and coregulator binding (Figure 3), it is clear that mutations in this domain will affect GR function extensively. Recently, a newly discovered point mutation in the LBD (V575G) was shown to disrupt multiple steps in the GC signaling cascade, including the affinity for the ligand, the time required for nuclear translocation, and the interaction with the GR interacting protein 1 coactivator, leading to decreased GC responses (147).

Importantly, polymorphisms located outside the coding region, eg, in introns, can also influence gene regulation by affecting gene splicing, transcription, and translation efficiency, or promoter activity, or by introducing a stop codon (148). For example, the so-called BclI site, located 647 bp downstream of exon 2, introduces a variant that cannot be cut by the BclI enzyme and is associated with GC hypersensitivity (128, 149, 150). Many studies have linked the BclI mutation with a wide variety of disorders, such as hypertension, adiposity, obesity (133, 151–153), and bulimia nervosa (154). Furthermore, BclI carriers are at risk of post-traumatic stress disorder (155), bronchial asthma (156), Crohn's disease (157, 158), cardiovascular disease (159), cystic fibrosis (160), obstructive airway disease (161), and atherosclerosis (153). In

Table 1. Mutations in the *NR3C1* Gene Observed in Humans

Polymorphism	GC Sensitivity	Increased Risk	Refs.
BclI	Increased	Hypertension Obesity Bronchial asthma Crohn's disease	151, 152, 156, 158
N363S	Increased	Obesity Bronchial asthma Type 2 diabetes Coronary artery disease	131–134, 136
ER22/23EK	Decreased	Rheumatoid arthritis Multiple sclerosis	137, 144
2314InsA	Decreased	Lupus nephritis	260
A3669G	Decreased	Primary myelofibrosis Diamond-blackfan anemia	80, 261, 262
TthIII I (together with ER22/23EK)	Decreased	Multiple sclerosis	144
A214G	Decreased	Septic shock	263
D401H	Increased	Hypertension Diabetes mellitus Accumulation of visceral fat	117
V423A	Decreased	Generalized GC resistance	145
R477H	Decreased	Generalized GC resistance Hirsutism Fatigue Hypertension	146, 264
T504S	Increased	Atopic dermatitis	260
T556I	Decreased	Generalized GC resistance Adrenocortical adenoma	265
I559N	Decreased	Cushing's disease Hypertension Oligospermia Infertility	266–268
V571A	Decreased	Generalized GC resistance	266, 269
V575G	Decreased	Hypertension Hypokalemia Oligoamenorrhea Generalized GC resistance	147
Δ612	Decreased	Familial GC resistance	270
D641V	Decreased	Generalized GC resistance Hypertension Hypokalemic alkalosis	266, 271, 272
S651F	Decreased	Atopic dermatitis	260
G679S	Decreased	Clinical GC resistance Hirsutism Fatigue Hypertension	146, 264, 273

(Continued)

Table 1. Continued

Polymorphism	GC Sensitivity	Increased Risk	Refs.
A714Q	Decreased	Generalized GC resistance Hypoglycemia Hypertension Clitoromegaly	274
V729I	Decreased	Generalized GC resistance Precocious puberty Hyperandrogenism	266, 275
F737L	Decreased	Hypertension Hypokalemia	276
I747M	Decreased	Generalized GC resistance Cystic acne Hirsutism Oligo-amenorrhea	70, 266
L753F	Decreased	Steroid resistant acute lymphocytic leukemia	100
L773P	Decreased	Generalized GC resistance Fatigue, anxiety Acne Hypertension	250, 252
F774S	Decreased	Hypoglycemia Hypertension Fatigue	252
9β	Decreased	Rheumatoid arthritis	137

contrast, carriers are less likely to develop RA (137) and osteoporosis (162).

Furthermore, variations in the 3' UTR region can influence the ratio of different GR splice variants because hGRα and hGRβ are generated by alternative splicing of the C-terminal exon 9 (48, 67). It has been reported that an A to G change at the first A in the ATTTA sequence resulted in stabilization of hGRβ mRNA and consequently increased hGRβ protein expression (76). This results in excess heterodimerization with hGRα and may reduce GR transcriptional activity (163). This might explain the increased risk of humans carrying this mutation for immune-regulated pathologies, such as myocardial infarction and cardiovascular disease (164).

VI. Post-translational Modifications of the GR Protein

The above-mentioned transcriptional and post-transcriptional regulation of GR illustrates the remarkable complexity of GR signaling. Although tissue specificity is determined primarily at the transcriptional level, each

isoform is subject to various PTMs, such as phosphorylation and SUMOylation. These covalent modifications of GR (Figure 4) affect its stability, subcellular localization, transcriptional activity, and interaction with other proteins. The modifications and their sites on the GR protein have been extensively studied during the past two decades.

A. Phosphorylation

The most common and best understood form of GR PTM is phosphorylation, which is the reversible attachment of a phosphate group to a protein, preferentially to serine (Ser) and/or tyrosine (Tyr) residues. GR was identified as a phosphoprotein more than two decades ago (165–170). The reversible phosphomodulation of GR is involved in modulating and fine-tuning the GC response. Phosphorylation of GR can affect GR ligand- and DNA-binding affinity, subcellular trafficking, and cofactor recruitment, culminating in altered transcriptional responses of GR, and consequently leading to a modified anti-inflammatory potential (171).

Phosphorylation is regulated by the balance between phosphorylating targeted kinases and dephosphorylating phosphatases. Six kinases that phosphorylate hGR have been identified: 1) the cyclin-dependent kinases (CDKs) (172–174); 2) the p38 MAPKs (175); 3) the c-Jun N-terminal kinases (JNKs) (176, 177); 4) the glycogen synthase kinase 3 β (GSK-3 β) (178, 179); 5) the ERK (172); and 6) casein kinase II (172). Several phosphatases that revert the phosphorylation have also been identified, including PP1, PP2a, and PP5 (180). These phosphatases are involved in the nuclear import of the ligand-activated GR (181–183).

Numerous phosphorylation sites on the hGR have been identified during recent years, including Ser113, Ser134, Ser141, Ser203, Ser211, Ser226, and Ser404, most of which are located in the AF1 domain in the NTD (172). These residues are conserved in homologous regions of other species, such as rat and mouse (184). The first identified residues showing enhanced phosphorylation in response to GCs are Ser203, Ser211, and Ser226 (185). In addition, mass spectrometric analysis of hGR

has identified other potential phosphorylation residues, ie, Tyr8, Ser45, Ser234, and Ser267 (186). However, it is still unknown whether these residues are actually phosphorylated.

Which kinases phosphorylate certain residues on the GR protein is not entirely known. Also, no GR-targeting phosphatases have been linked to specific GR residues so far. However, several studies have linked target residues with the executing kinase. For example, p38 has been shown to specifically phosphorylate Ser211, enhancing transcriptional and apoptotic activity (187, 188). Moreover, the site-specific phosphorylation of Ser211 by p38 leads to a modified, functionally active conformation of the AF1 domain that facilitates GR interaction with co-regulators and enhances subsequent GR transcriptional activity (189). However, when this Ser211 is targeted by other kinases, such as ERK and JNK, the transcriptional activity of GR is counteracted, compared to p38-induced phosphorylation of Ser211 (188). Ser211 has also been shown to be a target for CDK5 (27), but the outcome of the interaction is not clear (173). These data suggest that the effects of phosphorylation on GR activity are strongly dependent on the executing kinase. Ser203 has been shown to be the target of CDKs (A-CDK2, A-CDC2, B-CDK2, B-CDC2, and E-CDK2) and ERK kinases (190). Phosphorylation of Ser203 is required but is not sufficient for full GR activity because simultaneous phosphorylation of Ser211 is also required (191). Although the phosphorylation of Ser203 and Ser211 primarily enhances the transcriptional activity of GR, phosphorylation of Ser226 has been shown to decrease the activity, mainly by promoting the nuclear export of GR. Ser226 can be phosphorylated by JNKs and CDK5 (173, 176, 187, 192). Phosphorylation of Ser404 has been associated with GSK-3 β , and it leads to inhibition of GR activity due to increased nuclear export (178).

More recently, a new hormone-independent phosphorylation site, Ser134, was identified on the hGR; this site had already been picked up by mass spectrometry analysis (186). Phosphorylation of Ser134 is induced by the p38

MAPK in response to different stressors and by AKT (193). Furthermore, due to phosphorylation of Ser134, the GR shows increased association with the ζ -isoform of the 14–3–3 class of signaling protein (14–3–3 ζ), hence leading to increased transcriptional activity of GR (194). Binding of GR to 14–3–3 η is also known to enhance GR transcriptional activity (195), but whether this interaction also re-

Figure 4.

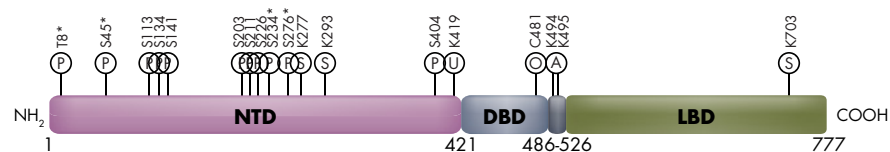


Figure 4. Post-translational modifications of the GR protein. General structure of the GR protein. Variation in the structure and function of the protein is partly caused by post-translational phosphorylation (P), SUMOylation (S), ubiquitination (U), oxidation (O), and acetylation (A). The asterisks refer to potential P-sites that have not been experimentally confirmed.

quires prephosphorylation of GR on Ser134 needs further investigation.

The phosphorylation status of GR has been linked to several disease states. For example, p38 MAPK has been shown to be active in alveolar macrophages of GC-resistant asthma patients (196). The Ser residue that is phosphorylated by p38 has not been identified, but speculations point to residue Ser211 or Ser226 (175, 188, 197, 198). Additionally, the phosphorylation status of GR, and specifically the MAPK-mediated abnormal phosphorylation of GR resulting in blunted GR activity, has been linked with disorders most often treated with GCs, such as leukemia, Crohn's disease, and asthma (197, 199, 200). On the other hand, overactivation of GR by increased phosphorylation at Ser211 has been linked with bipolar disorder, a severe mental illness (201).

Overall, it is clear that phosphorylation of GR has a major effect on its activity, suggesting that the GR phosphorylation status plays a critical role in the effectiveness of GC treatments. However, the concurrent phosphorylation sites and the accompanying kinases determine the specific outcome. For example, evidence shows that JNK-, ERK-, and GSK-3 β -mediated phosphorylation of GR blunts GR activity. These findings suggest that the concomitant administration of inhibitors for these kinases could abrogate the GC resistance observed in some disease states. Conversely, full GR activity requires the phosphorylation of several residues, such as Ser211. Both p38 and CDKs have been shown to enhance GR activity, primarily by targeting Ser211. Clearly, the phosphorylation of GR is another aspect of the complexity of its signaling.

B. Ubiquitination

Ubiquitination is an important PTM process that can be facilitated by precedent phosphorylation events and targets GR (202). Ubiquitination is the covalent attachment of multiple ubiquitin residues (8.5 kDa) by a series of ubiquitin enzymes (E-1 activating enzymes, E-2 conjugating enzymes, and E-3 ligases) to the target molecule. This labeling targets the protein for further processing, eg, proteasomal degradation by the 26S proteasome. E-2 and E-3 ubiquitin enzymes recognize a specific sequence on their target proteins, namely the PEST sequence (proline, glutamic acid, serine, threonine) (203, 204).

Ubiquitination and subsequent proteasomal degradation control the turnover of GR, and hence its transcriptional activity (205, 206). Furthermore, ubiquitination also increases the mobility of GR in the nucleus (206). This negatively affects the activity of GR due to induced GR export. Ligand-activated hGR becomes

ubiquitinated (K48-linked on residue lysine 419); this lysine is located in the degradation PEST motif in the NTD of GR (207).

GR interacts physically with the E-2 enzyme, UbcH7. Overexpression of UbcH7 reduces the transactivation potential of GR, whereas inhibition of the 26S proteasome by MG132 abolishes this reduction (208). GR is also regulated by three different E-3s, namely carboxy terminus of Hsp70-interacting protein (209, 210), ET-AP (210), and the human homolog of mdm2 (hmdm2) (211). Hmdm2 functions by generating a trimeric complex together with p53 (212).

Although ubiquitination can be cell-type specific, all known GR isoforms contain the target residue K419 and hence are subject to ubiquitin-mediated proteasomal degradation.

C. SUMOylation

Another PTM that can regulate GR function is SUMOylation, which is the addition of a small ubiquitin-related modifier-1 (SUMO-1) (213). SUMO-1 is a molecule of 11 kDa that is covalently attached to lysine residues of the target protein. Although SUMO-1 resembles ubiquitin in size and structure, SUMOylation does not directly target proteins for proteasomal degradation; it affects protein stability, protein-protein interactions, subcellular localization, and transcriptional activity (214, 215).

The hGR protein is thought to include three SUMOylation sites: K277, K293, and K703 (216). Overexpression of SUMO-1 destabilizes GR, but this can be reversed by inhibiting the proteasome. Furthermore, SUMO conjugase Ubc9 physically interacts with GR (217, 218). In contrast, a stimulatory effect of SUMOylation on the transcriptional activity of GR has also been reported (213). This might be due to SUMO affecting GR in a promoter- and cell-specific manner. However, a recent report demonstrates the interaction of RWD-containing SUMOylation enhancer (RSUME) with GR, which would increase its SUMOylation. RSUME positively regulates the transcriptional activity of GR by targeting K703, whereas targeting the other residues has a negative effect. Thus, the presence of both positive and negative SUMO-target sites in the GR could account for the conflicting data reported earlier (219). These SUMOylation sites have recently been shown to regulate the chromatin occupancy of GR on several loci. More specifically, SUMOylation seems to regulate GR activity in a target locus selective manner (220). Interestingly, SUMOylation of GR could also be interpreted as a phosphorylation-mediated event because phosphorylation of GR Ser226 by JNK facilitates the SU-

MOylation of GR at its N-terminal SUMOylation target sites (221).

D. Acetylation

GR protein function is also under the control of acetylation. Although enzymes involved in (de-)acetylation events, ie, histone acetyltransferases and histone deacetylases (HDACs), primarily target the lysines located in histone tails, there is evidence for direct acetylation of other proteins, notably GR.

Before nuclear translocation, GCs induce acetylation of GR at K494 and K495, located in the hinge region (176). A more recent report points to acetylation of a cluster of lysines in the hinge region by the circadian rhythm-generating transcription factors CLOCK and BMAL1, thereby diminishing the transcriptional activation of a GRE reporter by GR (222). Moreover, to interact with NF κ B and repress inflammation, GR must be deacetylated by HDAC2 (176). Several studies have shown that overexpression of HDAC2 is involved in the reversal of GC resistance in patients with chronic obstructive pulmonary disease and asthma (223–227). We speculate that hyperacetylation of GR in the absence of HDAC2 or due to high levels of CLOCK and BMAL1 leads to GC insensitivity in patients.

E. Nitrosylation

Nitrosylation has been reported to affect GR activity. Several critical cysteines, which are necessary for ligand binding, are located in the DBD and LBD of GR (226, 228). Generally, cysteines contain sulfhydryl groups, which are extremely prone to react with nitric oxide (NO) to form S-nitrosothiols. Besides the early identification of Cys656 as one of the first amino acids to be modified of the GR (229), no additional specific cysteines have been designated to be targeted by nitrosylation. Nevertheless, the use of NO donors could decrease the binding of GR to its ligand, and this binding could be inhibited by the thiol-protecting agent dithiothreitol (230). Moreover, NO interferes with the GR-mediated anti-inflammatory effects of GCs. Therefore, nitrosylation of critical sulfhydryl groups in GR by NO could be the reason for GC insensitivity of septic shock patients, who have higher levels of NO (226). Next, inhibiting neuronal NO synthase in the hippocampus increased GR expression, indicating that neuronal NO synthase is an inhibitor of GR expression in the hippocampus, and therefore a potential modulator of the entire hypothalamic-pituitary-adrenal gland-axis cascade (231, 232).

However, the effect of NO on GR activity remains controversial. Other studies have reported that GR expression is up-regulated by NO and suggested that GC therapy

could be more effective in combination with NO (233). Furthermore, exogenous NO has been shown to activate the endothelial GR, and specifically its nuclear translocation and the transactivation of reporter constructs (234). In addition, GR-mediated limitation of inflammation could be potentiated by a NO-donating prednisolone derivative, NCX-1015. This was assessed by augmented binding to its ligand, nuclear translocation, and the rapid inhibition of neutrophil recruitment in a model of peritonitis (235). Obviously, the effect of nitrosylation on the GR protein, whether it is stimulatory or inhibitory, requires more in-depth investigation.

F. Oxidation

Studies have shown that the functional activity of GR can be suppressed by oxidative conditions and restored in the presence of reducing agents (236, 237). Oxidation is closely associated with nitrosylation because it also mainly targets sulfhydryl groups. Cysteine 481 (Cys481) in particular seems to be a target for oxidation because its substitution reduced the sensitivity of GR to oxidative treatments, such as H₂O₂. Modification of GR by oxidation has been shown to reduce ligand binding and subsequent DNA binding activity of GR. Reducing agents, such as dithiothreitol or N-acetyl-L-cysteine, and the overexpression of thioredoxin reductase, effectively counteract the negative effect of H₂O₂ (169, 226, 236, 238–241). Oxidative stress can also indirectly influence GR function by decreasing the expression and activity of HDAC2, which has been linked with GC insensitivity in asthma patients (223, 242).

VII. Role for DNA Binding Sequences in Directing GR Conformation and Function

GR binding sequences (GBSs) are abundant in the mammalian genome (Figure 5). However, recent genome-wide ChIPseq analyses have shown that only a fraction of the GBSs are bound by GR (243–245). These sequences are typically imperfect palindromic hexamers separated by a 3-bp spacer. The first hexamer consists of five positions that are nearly identical in different species and among the promoters of different GR-inducible genes (246). The spacer and second hexamer are less conserved. Recent research indicates an important, formerly unidentified, role for the DNA binding sequence in instructing GR-mediated transcriptional activation. The first evidence came from Meijnsing et al (85), who illustrated that the DNA binding sequence forms an allosteric ligand with GR, directing minor but important changes in the receptor's structure and hence in transcriptional activity. That study demon-

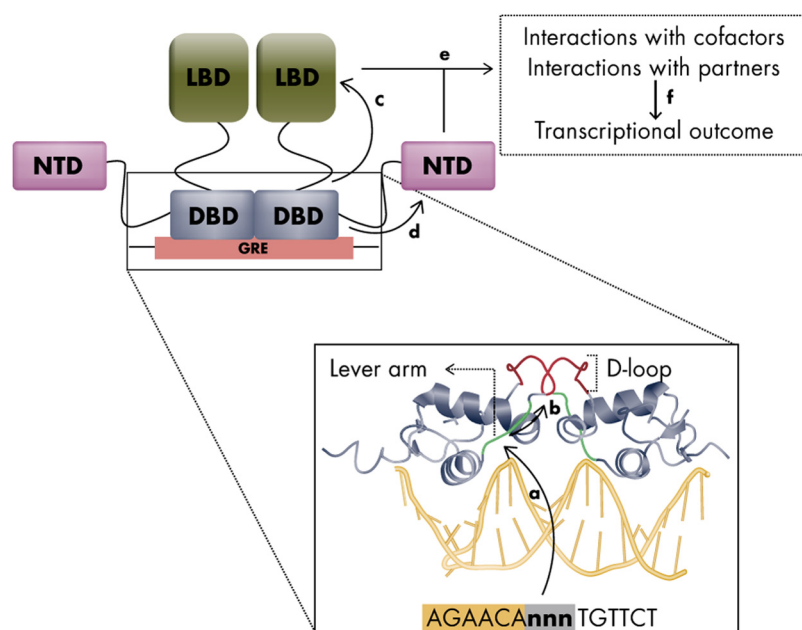
Figure 5.

Figure 5. DNA binding sequence directs GR structure. General overview of the GR protein binding to its GRE, zoomed in on the DBD. a, Upon binding to the first half-site (yellow box), the spacer sequence (gray box) directs the conformation of the lever arm. b, The lever arm subsequently translates this information to the D-loop in the DBD. c and d, The DBD conformation directs the structure of both the LBD and NTD, respectively. e and f, This further influences the interactions with cofactors and other partner, and hence the transcriptional outcome of GR signaling.

strated that GBSs use GR surfaces, ie, the AF1 and AF2 domains and the dimerization loop (D box) in the DBD, in different ways. For example, GBSs with identical half-sites but different spacers direct the different usage of these three GR surfaces. Interestingly, a crucial role was described for the “lever arm,” a domain within the DBD that connects the DNA-binding loop (P box) with the dimerization loop (D box). This observation was supported by a recent study by Watson et al (108), who provide evidence that the interaction with bases at particular positions in the binding sequence, such as in the first half-site or in the spacer region, directs the conformation of these distinct GR regions. The authors suggested that the DNA sequence tailors the conformation of the lever arm. Extra evidence for the crucial role of the lever arm was provided by using the DBD of the GR γ isoform, which has an extra arginine residue in the lever arm. This isoform and the GR α isoform up-regulate different sets of genes. The DNA occupancy and structures, however, were comparable for both isoforms, suggesting that the lever arm directs gene-specific events after GR-DNA binding and transmits the signals to other GR domains (85). More recently, it was demonstrated that the selection of GR binding regions by the GR γ isoform and the subsequent downstream events are

directed by the γ insertion in the lever arm (108, 247). Furthermore, it was suggested that the lever arm of GR mediates bidirectional allosteric signaling. DNA sequence selectivity is translated into conformational changes in the DBD, more specifically the D-loop, and changes in the dimerization domain affect other GR surfaces that can interact with cofactors (247). Proof of concept has been provided by mutating the dimer interface, which resulted in an altered sequence-specific conformation of GR, DNA-binding kinetics, and transcriptional activity. Moreover, it was illustrated that GR dimer partners collaborate to interpret the DNA shape and sequence in order to direct the sequence-specific gene transcriptional activity of GR (108). In conclusion, GBSs seem to direct the structural changes at the DNA binding interface, which is translated by the lever arm to the dimerization interface and GR dimer partners, and hence results in differential transcriptional outcomes. The mechanism regulating DNA-binding kinetics and the effects on the transcriptional outcome of GR remains to be elucidated. However, it has been proposed that the width of the minor groove might act as an indirect readout of spacer sequence (108).

A crucial role has also been shown for the nGRE consensus sequence, CTCC(N)₀₋₂GGAGA, in controlling GR binding orientation and dimerization (248). These so-called negative GREs were thoroughly described by Surjit et al (249) and shown to be present in over 1000 mouse/human orthologous genes. GR monomers occupy the DNA half-sites of these nGREs, but they do not homodimerize (248). Moreover, it was suggested that these nGREs affect the conformation of some critical GR residues, which are critical for transcriptional activation, hence resulting in repression instead of activation (248). Hence, these findings support the observation that DNA is an allosteric modulator of GR activity. Overall, the above-mentioned findings show that the DNA sequence acts as a modulator affecting the interaction of GR with DNA and cofactors by directing conformational changes in its dimerization interface, and hence directing the transcriptional outcome.

VIII. Future Perspectives and Therapeutic Implications

GCs are widely used to treat many inflammatory conditions, but the outcome varies considerably among patients. This variation can be attributed to the presence of different GR isoforms with unique expression and gene regulatory profiles or different polymorphisms in the hGR gene. The recent finding that the GR-bound DNA sequence acts as an allosteric ligand directing the activity of GR can also account for the gene-, tissue-, and individual-specific effects observed. Furthermore, not only the DNA sequence, but also epigenetic regulators, context, and other unrecognized regulatory factors can influence the transcriptional outcome, hence complicating the picture of GR function and regulation. However, development of new revealing techniques, such as ChIPseq and nuclear magnetic resonance, will help uncover the genome-wide profiling of cistromes and the structural biology of transcription factors (14, 15, 108, 247, 251). Hence, determining the presence of these hGR variants and regulatory factors in a patient is critical for optimizing GC dose for maximal therapeutic efficacy and minimal side effects. To develop personalized GC-based therapy, future research should also focus on the processes that mediate the generation of GR splice variants and translational isoforms. Obviously, the research community still lacks detailed knowledge on the mechanisms of GR structure and function. Structural and functional studies of other nuclear receptors could also help us to look at GR from a new angle (reviewed in Ref. 253).

Reduced GC sensitivity can also be caused by the pathophysiological processes. It is well known that a proinflammatory environment can negatively affect GR function in many ways (254, 255). Because various cell types and cytokines are involved in the pathogenesis of inflammatory diseases, the mechanisms contributing to decreased GC sensitivity in any particular disease are heterogeneous. However, the mechanisms of reduced GC responsiveness are not well understood, although numerous mediators have been identified. For example, next to the critical role for miRs, a role for small noncoding RNAs was recently revealed. Noncoding RNA *Gas5*, which is associated with growth arrest and starvation, was shown to repress GR transcriptional activity. *Gas5* can bind specifically to the DBD of GR by acting as a decoy GRE, and thereby compete with GR for binding to its GREs (256). Thus, a more detailed understanding of the molecular mechanisms of GR function may reveal new drug targets that could be exploited to sensitize resistant diseases to the anti-inflammatory effects of GCs. Furthermore, cell or tissue-based therapies could be used, such as GCs delivered in lipo-

somes, topical application of GCs, and targeted delivery to the colon of dextran and sulfonate conjugates (257–259) to increase GC effectiveness and reduce potential adverse side effects. A combinatory therapeutic approach can also be considered. GCs could be combined with a substance inhibiting a PTM of GR. Such a substance could be a downstream molecule of the signaling pathway of various cytokines because there is an intricate interplay between GR and cytokines, such as TNF and IL-1 β (reviewed in Refs. 254 and 255). The use of such combined approaches should allow the use of lower doses of GR ligands, which would reduce the side effects and increase sensitivity. Finally, the current and future findings on GR function should be translated to the clinic to ensure more effective and safer GC therapies.

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